NO 5113

# INTER LIBRARY LOAN REQUEST FORM

CMI - 7E03

Borrower's Name: Chris Chin | Org. or A.U.: 1641 | Phone: 308-3991

Serial Number: | Date of Request: 5/12/03 | Date Needed By:

Please Attach Copy Of Abstract, Citation, Or Bibliography, If Available. Please Provide Complete Citation. Only One Request Per Form.

Author/Editor: T. K. Roberts, R. Price, Y. C. Smart, K. Stevenson

Journal/Book Title: Reproductive & Perinatal Medicine

Article Title: Early Pregnancy Factor of human urine 4455 (

Volume (Issue): 1 ( Early Pregnancy Factors)

Pages: 191 - 197

Year of Publication: 1985

Publisher:

Remarks: 10549787

AN - 105:112770 CA

STIC Use Only

Accession Number: _____

| LIBRARY ACTION | LC | | NAL | | NIH | | NLM | | NBS | | PTO | | OTHER | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Local Attempts | | | | | | | | | | | | | | |
| Date | | | | | | | | | | | | | | |
| Initials | | | | | | | | | | | | | | |
| Results | | | | | | | | | | | | | | |
| Examiner Called | | | | | | | | | | | | | | |
| Page Count | | | | | | | | | | | | | | |
| Money Spent | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

Provided By: Source and Date

Ordered From: Source and Date

Remarks/Commer

1st & 2nd denotes time taken to a library

O/N - Under NLM means Overnight Service

FX - Means Faxed t us

© Perinatology Press, 1985
EARLY PREGNANCY FACTORS
F. Ellendorff and E. Koch, eds.



# EARLY PREGNANCY FACTOR OF HUMAN URINE

T.K. ROBERTS, R. PRICE, Y.C. SMART, K. STEVENSON, V. TASEVSKI
University of Newcastle, N.S.W. 2308, Australia.

In recent years, reports of the isolation and purification of mammalian early pregnancy factor (EPF) have revealed that the factor is associated with proteins of relatively low molecular weight. EPF from sheep serum was found to exist as an entity of 20KD (3) and mouse EPF 21KD (1). We have undertaken studies on human EPF using as source material urine from pregnant women.

Initial studies confirmed that urine could be used as a source of EPF. All urine samples tested from women pregnant up to 28 weeks were positive for EPF in the rosette inhibition test. The rosette inhibition titres (RIT) were determined as described previously (2). Pooled urine was fractionated by cation exchange chromatography (CM-Sephadex, 0.05 M acetate pH 4.9). Using a linear salt gradient EPF activity was routinely found in the 220 mM to 225 mM NaCl fraction. This concentrated material presented no bands on silver staining following detergent-electrophoresis on polyacrylamide gels. The EPF fraction was then used for antibody production and further characterisation.

Interesting results were obtained with dialysis and ultrafiltration. Using both this fraction and whole urine EPF activity was not lost following dialysis, however, when subjected to serial ultrafiltration (50,000 Daltons exclusion, followed by 25,000 Daltons; followed by 10,000 Daltons) EPF activity was detected in the greater than 50,000 Daltons fraction and in the fraction between 3,500 and 10,000 Daltons (Table 1). The low molecular weight fraction obtained by ultrafiltration was dialysable. Further studies showed that on mixing the low molecular weight dialysable form with an EPF-negative 10,000 to 25,000 Daltons fraction from non-pregnant urine, the EPF activity was no longer dialysable. It was concluded that in pregnant urine EPF existed as a low molecular weight entity bound to a larger carrier.

TABLE 1. EPF ACTIVITY OF PREGNANT URINE AFTER SERIAL ULTRAFILTRATION

| Molecular Weight | RIT | EPF |
|---|---|---|
| Greater than 50,000 | 64 | + |
| Less than 50,000 | 256 | + |
| Between 25,000 and 50,000 | 8 | - |
| Less than 25,000 | 256 | + |
| Between 10,000 and 25,000 | 8 | - |
| Less than 10,000 | 256 | + |

The purified EPF obtained from the CM-Sephadex column was used for the generation of polyclonal antisera and monoclonal antibodies. Two different rabbits produced antisera capable of inhibiting EPF activity as seen in the standard rosette inhibition assay. Similarly two of the monoclonal antibodies produced were able to block EPF activity of pregnant serum. The RIT values obtained using one of the positive monoclonals (2F11) and a negative monoclonal (AMT) are presented in Table 2. The monoclonal antibody 2F11 was further shown to inhibit the EPF activity present in pregnant urine; the purified fraction obtained from CM-Sephadex; the greater than 50,000 Dalton fraction of pregnant urine; and the less than 10,000 fraction of pregnant urine.

TABLE 2. EFFECT OF MONOCLONAL ANTIBODIES OF EPF ACTIVITY

| Treatment | | | |
|---|---|---|---|
| Antibody | Source of CM-Sephadex Fraction | RIT | EPF |
| - | Pregnant urine | > 128 | + |
| - | Nonpregnant urine | 16 | - |
| 2F11 Supernatant | Pregnant urine | 16 | - |
| 2F11 Supernatant | Nonpregnant urine | 16 | - |
| AMT Supernatant | Pregnant urine | > 128 | + |
| AMT Supernatant | Nonpregnant urine | 16 | - |
| 2F11 Ascites | Pregnant urine | 16 | - |
| 2F11 Ascites | Nonpregnant urine | 8 | - |
| AMT Ascites | Pregnant urine | 128 | + |
| AMT Ascites | Nonpregnant urine | 16 | - |

REFERENCES

1. Cavanagh, A.C. factor and pur
2. Smart, Y.C., R R.L. (1982) Va detection of e
3. Wilson, S., Mc pregnancy fact from pregnant

FTER SERIAL ULTRAFILTRATION

| RIT | EPF |
|---|---|
| 64 | + |
| 256 | + |
| 8 | - |
| 256 | + |
| 8 | - |
| 256 | + |

Sephadex column was used for
noclonal antibodies. Two
of inhibiting EPF activity
ssay. Similarly two of the
block EPF activity of pregnant
the positive monoclonals
resented in Table 2. The
o inhibit the EPF activity
tion obtained from CM-
ction of pregnant urine; and
ine.

DIES OF EPF ACTIVITY

| Fraction | RIT | EPF |
|---|---|---|
| | > 128 | + |
| ne | 16 | - |
| | 16 | - |
| ne | 16 | - |
| | > 128 | + |
| ne | 16 | - |
| | 16 | - |
| ne | 8 | - |
| | 128 | + |
| ne | 16 | - |

REFERENCES


1. Cavanagh, A.C. (1984) Production in vitro of mouse early pregnancy factor and purification to homogeneity. J. Reprod. Fert. 74, 581.
2. Smart, Y.C., Roberts, T.K., Fraser, I.S., Cripps, A.W. and Clancy, R.L. (1982) Validation of the rosette inhibition test for the detection of early pregnancy in women. Fert. Ster. 37, 779.
3. Wilson, S., McCarthy, R. and Clarke, F. (1984) In search of early pregnancy factor: characterisation of active polypeptides isolated from pregnant ewe's sera. J. Reprod. Immunol., 6, 253.